1270883

THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE

United States Patent and Trademark Office

January 07, 2005

BEST AVAILABLE COPY

THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A FILING DATE.

APPLICATION NUMBER: *60/528,410*
FILING DATE: *December 10, 2003*
RELATED PCT APPLICATION NUMBER: *PCT/US04/41923*

UNITED STATES PATENT AND TRADEMARK OFFICE
DEPARTMENT OF COMMERCE

Certified By

Jon W Dudas

Under Secretary
of Commerce for Intellectual Property
and Acting Director of the
Unites States Patent and Trademark Office

Rev 9/03

PROVISIONAL APPLICATION COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION under 37 CFR 1.53 (c).

158896 U.S.PTO
22151 U.S.PTO 60/528410
121003

MAIL STOP PROVISIONAL APPLICATION
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Docket Number: NU-642Xq800	Type a Plus sign (+) inside this box →	+

INVENTOR(s)/APPLICANT(s)

LAST NAME	FIRST NAME	MIDDLE INITIAL	RESIDENCE (CITY AND EITHER STATE OR FOREIGN COUNTRY)
Kautz	Roger	A.	79 Little Nahant Road, Nahant, MA 01908
Karger	Barry	L.	62 Deborah Road, Newton, MA 02159

[ ] Additional Inventors are being named on Page 2 attached.

TITLE OF THE INVENTION (280 characters max)

METHOD FOR EFFICIENT TRANSPORT OF SMALL LIQUID VOLUMES IN MICROFLUIDIC DEVICES

CORRESPONDENCE ADDRESS

[X] Customer Number 207

which is associated with the Law Firm of:
WEINGARTEN, SCHURGIN, GAGNEBIN & LEBOVICI LLP
Ten Post Office Square
Boston, Massachusetts 02109
United States
Telephone: (617) 542-2290 Fax: (617) 451-0313

ENCLOSED APPLICATION PARTS *(CHECK ALL THAT APPLY)*

[X] Specification incl. Figs. Number of pages [22]

[ ] Drawing(s) Number of sheets [ ]

[X] Small Entity status is entitled to be, and hereby is, asserted for this application

[ ] Other (specify)

METHOD OF PAYMENT *(CHECK ONE)*

[X] A check in the amount of $80.00 is enclosed to cover the Provisional Filing Fee

[ ] The Commissioner is hereby authorized to charge filing fees and credit Deposit Account Number 23-0804

Please recognize the following attorneys with powers in this application.

Stanley M. Schurgin, Reg. No. 20,979
Charles L. Gagnebin III, Reg. No. 25,467
Victor B. Lebovici, Reg. No. 30,864
Beverly E. Hjorth, Reg. No. 32,033
Holliday C. Heine, Reg. No. 34,346
Gordon R. Moriarty, Reg. No. 38,973
James F. Thompson, Reg. No. 36,699

Respectfully submitted,

SIGNATURE Holliday C. Heine DATE Dec. 10, 2003

TYPED or PRINTED NAME: Holliday C. Heine, Ph.D. REGISTRATION NO. 34,346

PROVISIONAL APPLICATION FILING ONLY

Express Mail No: EV044750303US
HCH/raw 299298-1

This Page Is Inserted by IFW Operations
and is not a part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

As rescanning documents *will not* correct images, please do not report the images to the Image Problem Mailbox.

INVENTION TITLE:

Method for Efficient Transport of Small Liquid Volumes in Microfluidic Devices

INVENTORS:

Roger A. Kautz 566-82-8636 US Citizen NU Employee

Barnett Institute (617) 373-8211

Barry L. Karger US Citizen NU Employee

Barnett Institute (617) 373-4816

DESCRIPTION

This invention is a method of moving small samples through the capillary channels or tubing of a microfluidic device without dilution of the sample or loss to the capillary wall. The sample is a small volume of liquid, for example a solution of an analyte for chemical analysis. In this method the microfluidic device is filled with a liquid which is not miscible with the sample, which we will call the immiscible carrier liquid. When the sample is introduced into the immiscible carrier liquid, the sample forms a segment or "plug" in the microfluidic channel or capillary. When the carrier liquid is pumped or otherwise caused to flow through the channel, the sample is carried from one location to another through the microfluidic channels without dilution or dispersion into the immiscible carrier liquid. In particular, if the carrier liquid has a lower contact energy with the wall of the channel, a film of the carrier liquid will wet the channel wall as the sample plug passes, so that the sample plug will not contact the channel wall. This avoids losses of analyte either by binding of analyte molecules to the capillary wall or by bulk loss of sample as a film on the channel wall. Small liquid samples may thus be transported long distances through microfluidic plumbing with very low losses, and at relatively high speed. As a specific example, if the immiscible carrier liquid is a fluorocarbon (FC), and the channel surface is fluorine-rich, the carrier liquid will wet the channel wall preferentially to both aqueous and organic (hydrophobic and hydrophilic) solvent samples. The desired effect may be obtained by either making the channel of a

Express Mail Number
EV044750303US

fluorine-rich material such as a teflon (PTFE, ETFE, FEP, NGFP, etc.) or the channel wall may be coated with a fluorine-rich layer such as a fluoroalkyl silane coating on glass, silica, or plastic.

This invention has been embodied in a method for loading samples into the detection cell ("probe") of a Nuclear Magnetic Resonance (NMR) spectrometer. So-called microcoil NMR probes have recently been introduced, offering a 10 to 50-fold advantage in mass sensitivity over conventional probes but posing a problem in transferring small concentrated samples into the active volume of the NMR detection cell. The detection cell is recessed 50 cm or more up the narrow bore of the NMR magnet (Fig. 1). In addition, any motorized equipment such as pumps or autosampler must be located outside of the fringe field (5 gauss line) of the magnet, necessitating an additional 1-10 meters of capillary tubing, depending on the magnet strength and design. This present invention enables the transfer of small concentrated samples (e.g. 1 uL, 1 mM) from an automated sample handler through such lengths of capillary tubing into the NMR probe, with less than 5% loss or carryover. Significantly, it permits multiple sample plugs to be injected at closely-spaced intervals, enabling much faster sample changes. Additionally, it eliminates "relaxation delays" conventionally required after injection of each sample before quality spectra could be obtained.

- Former Approaches and Disadvantages.

Conventionally, samples for NMR are prepared in high-precision 5 mm diameter glass tubes, which are inserted parallel to the bore of the magnet into an NMR detection coil (radiofrequency antenna) of a 'saddle coil' design, which is open along the axis of the magnet to accommodate the insertion of tubes (Fig 2A).

A recent adaptation has been to replace the interchangeable tubes with a fixed flow cell (Fig 2B), which may be filled and emptied through tubing. Originally marketed to use the NMR spectrometer as an on-line detector for liquid chromatography (LC-NMR), this flow-probe design has found strong application as a means to load samples using a robotically controlled syringe ('sample handler' - Figs. 1 and 3). Two strengths of such

'direct injection NMR' or 'flow-injection NMR' methods (Keifer, 2000; Keifer, 2003) are: (1) samples can be drawn directly from a variety of standard sample containers, including microtiter well plates, without first being transferred to NMR tubes; and (2) the system allows higher throughput when many similar samples are to be analyzed. The conventional flow probes are based on the large open saddle-coil design used in probes for NMR tubes. The saddle coil is open along the magnet axis permitting tubes to be inserted, but is not the most sensitive design.

Significant sensitivity advantages of 10-50-fold were realized by making the NMR detection coils (a) smaller, and (b) of a solenoid design (Fig. 2C) (Lacey, 1999). The solenoid, however, must be oriented transverse to the field (the magnet axis) making it impractical to insert sample tubes perpendicularly to the narrow magnet bore. One approach, used in 'solids' probes, is to remove the probe from the magnet to change samples. A liquids NMR system, to be commercially competitive, must be able to change samples rapidly and be automatable.

Microcoil NMR probes are thus plumbed as flow cells, communicating to outside the magnet through capillary tubing. However there are several inherent challenges in transferring a small concentrated sample, for example 1 ul or less, through such a capillary flow system. Filling the entire plumbing with sample solution is grossly inefficient,. for the probe shown in Fig. 2C less than 0.3% of the sample resides in the NMR coil detection volume, defeating the sensitivity advantages of the microcoil. A small volume sample might be moved through the capillary using either air pressure or a carrier solvent. Samples in air would paint the wall of the capillary and be lost; it would be difficult to position the sample accurately in the coil region; and it has been shown that samples bounded by air must be at least seven times longer than the coil, which is an inefficient use of limited sample material. Driving short sample plugs with clean solvent as a carrier fluid is not feasible because the sample mixes with the solvent in the capillary tubing and becomes more dilute. The increase in length of a sample plug is nearly equal to the distance it is moved, because the fluid contacting the capillary wall is essentially stationary. It should be noted that the time required to acquire an NMR spectrum increases as the inverse square of the concentration: a 10-fold dilution requires 100 times longer to acquire similar quality data. We previously published a method for

electrophoretically concentrating dilute samples *in situ* into the NMR coil (Kautz, 2001), but it is not applicable for all analytes and requires hours or days per sample.

An approach used in a commercial microcoil NMR system is to use the smallest bore capillary possible for the transfer line, feeding into an enlarged flow cell at the NMR coil detection region (Fig 2D). The practical lower limit on the transfer line size (inner diameter) is that the pressure required to transfer the sample within a few minutes must not exceed several thousand pounds per square inch (psi), the pressure attainable with high pressure liquid chromatography (HPLC) pumps and capillary tubing fittings. Typically 50 um capillary is used, which has a volume of 2 uL/meter. Samples of 1 uL or smaller will still show dispersion in the lines. Although microcoil probes smaller than 1 uL (e.g. Fig. 2C) are both practical and more sensitive (Lacey, 1999: Kautz, 2001), the commercial microcoil probe uses a 1 uL NMR coil detection volume (3.5 uL flow cell), which is a compromise with this limitation. A second limitation is that pumping solvents through 50 um capillaries requires specialized high-pressure pumps, tubings, and plumbing connections, including the use of a sample loop and switching valve between the low pressure injection port at the sample handler and the high pressure transfer line to the NMR detection cell (Fig 4). These components add significant cost and complexity, and can be problematic in use.

There is also substantial dilution in the flow cell (Fig. 5A). The flow cell volume must be several times the volume of the NMR detection coil, because high-quality NMR spectra can only be obtained when discontinuities in the magnetic properties between the ends of the sample and its container are remote from the NMR coil (infinite cylinder approximation). Typically the flow cell volume must be 3 times the NMR coil volume. The leading edge of the sample, as it is introduced into the flow cell, will mix with and dilute into the larger volume of the flow cell. In practice, any sample less than the flowcell volume will be diluted into the flowcell volume or more (Keifer, 2003).

Another throughput limitation in the current implementations of flow-injection NMR is that after loading a sample into the flow cell the sample must 'relax' for a period of time

before high quality NMR spectra can be obtained (Keifer, 2003). Typically, immediately after introduction the resolution (linewidth - full-width-at-half-max) of the spectrum will be 3-5 hz. This will decrease over 2-5 minutes to below 1 hz, similar to the optimum value obtainable from a uniformly-filled flow cell. This 'relaxation' or 'equilibration' effect has been attributed to the slow diffusion and smoothing of the strong analyte concentration gradient within the flow cell from the dilution on injection described above.

And finally, many analytes bind to silica, a preferred material for capillary tubing.

- Limitations of conventional art:

Small samples are diluted to at least flowcell volume.

Relaxation time after injection. On-flow performance (LC-NMR) compromised.

High pressure requires additional equipment.

High pressure system susceptible to failures in plumbing:

Connections leak, port seals fail, components clog.

– Novel and Unusual Features of the Present Invention

An immiscible carrier fluid is used for transfer to avoid dilution (Fig. 5B).

The immiscible carrier may be a fluorocarbon liquid, which is immiscible with both aqueous and organic solvents; apparently all solvents and analytes other than mixed hydrocarbon - fluorocarbon solvents.

The transfer path is made of a perfluorinated or highly fluorinated material, or is coated with such a material, so that the channel surface is preferentially wetted by the fluorocarbon solvent. In this case a film of the fluorocarbon liquid is maintained between the channel wall and the sample as it passes, such that the sample for analysis does not contact the channel wall. This prevents adsorption of the analyte to the wall directly, or bulk loss of the analyte solution to film formation on the channel wall (Fig. 6) (Curcio, 2003; Nord & Karlberg, 1984).

The above features permit efficient transfer of small discrete samples for analysis through the microfluidic system, and show a marked advantage to uniformly filling the channels; to injecting plugs of sample in clean sample solvent as a carrier fluid; or to using an immiscible organic solvent as carrier liquid.

-- Advantages

Sample Efficiency. Trace samples may be analyzed. No sample is wasted. Use of sample may be 100% efficient, as opposed to 10-30% (in commercial Protasis/MRM microinjection) or 0.1% (filling a 200 um microcoil probe). Figure 7 shows that there is no degradation of sensitivity or resolution if sample plugs as small as 1 uL are picked up by the autosampler and transferred into an NMR probe with a 3.5 uL flowcell/ 1 uL observe volume.

Rapid Sample Changes Along a Queue of Sample Plugs. (Fig. 8) Conventionally, a sample loaded in the sample loop must be delivered the entire distance to the NMR coil (Figs. 3,4). With the immiscible plug method, small sample plugs may be closely spaced, separated by plugs of the immiscible solvent (segmented flow injection). For example, if 1 uL sample plugs are separated by 0.5 uL of immiscible , samples may be changed by moving the queue only 1.5 uL. Figure 9 shows an implementation of this method which has demonstrated automated unattended acquisition of consecutive trains of multiple sample plugs.

Rapid Washing of NMR Detection Cell In conventional DI-NMR and FIA-NMR methods the flowcell must be flushed with several volumes of clean solvent between samples to reduce sample carryover. With the present invention, one sample plug may be followed closely with one or more small plugs of clean solvent to rinse any traces of sample from surfaces or dead volumes of the plumbing. This 'train' of sample and rinse plugs may be less than 2 uL and subsequent samples may follow immediately, in a flow-through injection scheme.

No Relaxation Time Required after Sample Injection. Figure 9 shows linewidths of spectra as a sample plug is passing on-flow through the NMR coil. Because the sample plug is of uniform concentration, there are not strong concentration gradients as in the conventional methods, so the linewidth of the NMR spectrum is sharp immediately upon arrival in the NMR coil.

Sample Recovery. Because samples are maintained in their original volume of 1-2 uL, sample recovery is greatly facilitated. The photograph shown in Figure 6B is in fact of a sample plug after passage through a microcoil NMR probe. With a miscible carrier liquid, analytes disperse over a volume of 5-20 uL. Consequently and in addition, the leading and trailing edges of the resulting analyte zone are not well-defined, and are difficult to detect. With immiscible solvent plugs the sample zone is sharply defined, and can be detected by the physical properties of either solvent or of the sample itself or of the sharp boundary, such as UV or visible absorbance, conductance, viscosity, light scattering, surface tension, or others. A conventional liquid chromatography fraction collector may be used. Alternatively, simply placing a length of teflon tubing on the outlet capillary of the NMR probe collects the sample and wash plugs, which may be discerned by eye.

Accurate Positioning of Sample Plugs in the Detection Cell. The diffuse leading and trailing edges of sample plugs in the conventional methods make it difficult to determine the optimal positioning of the sample in the NMR cell. As is seen in Figure 10, with the immiscible plug method the leading edge of the sample plug is sharp and easily detected; the plug can be accurately positioned by timing from the arrival of the leading edge. This method has been implemented.

Samples may be transferred in larger bore capillary tubing, reducing backpressure and consequent need for specialized pumps and related plumbing equipment.
Samples may be transferred over longer distances without loss. High-end NMR spectrometers have larger magnets with larger fringe fields, which may require the

sample handler to be as far as 10 meters. With the present invention there is no disadvantage in sample efficiency or throughput with longer transfer line lengths. Larger capillaries permit fast transfers even over such distances, where 50 micron capillaries would be prohibitive.

Stability for Long Acquisitions. While basic "One-Dimensional" NMR spectra are generally acquired from concentrated samples in a few minutes or less, more information or more dilute samples can require NMR acquisition times of several days or more. Miscible solvent plugs can diffuse out of the detection volume over such long periods of time. The immiscible solvent plugs are stable indefinitely. (Plugs stored in teflon tubing have remained intact and undiluted for over a year.)

– Alternative or Improvements Present or Planned

Improved coatings for standard capillaries. The efficient sample transfer by the immiscible plug method depends on the relative contact energies of the sample solvent and immiscible carrier solvent with the channel wall. These contact energies can be modified by chemically modifying the channel wall. Figure 11 shows sample plugs of DMSO-d6 (with blue dye) separated by a fluorocarbon liquid (clear), in teflon, fused silica, and perfluoroalkyl silane-treated fused silica capillaries (FAS-silica). The fused silica capillaries are mechanically more rigid, more suitable for higher pressures, and easier to make high-pressure connections. FAS-silica would enable the existing commercial microcoil probes to use the immiscible plug injection method, because the silica can tolerate the back-pressures generated when driving flow through their 50-micron capillaries.

Smaller microcoil probes. The current (and only) commercial microcoil NMR probe has an NMR coil detection volume ("active volume", "observe volume", V_{obs}) of 1 ul, designed based on the typical size of a capillary LC peak, or of the smallest sample that can be injected using a commercial autosampler, considering the limitations of dilution

during transfer. With the present invention of Immiscible Solvent Plug Injection, samples of arbitrarily small volume may be efficiently transferred into smaller microcoil probes. In particular, the most sensitive microcoil NMR probes produced to date are wrapped directly on 200/360 um (i.d/o.d.) capillaries and have an observe volume of 30 nL, but sample transfer into the coils is difficult. (ref Kautz *et al.* 2001 cITP-NMR). With the present Immiscible Solvent Plug Injection method, samples of approximately 30 nL volumes can be efficiently transferred, making these smaller probes, which are three times more sensitive, feasible for routine samples or high-throughput use.

Different Carrier Liquids. The above work was performed using fluorocarbon FC-43 as the immiscible carrier liquid. Many other immiscible solvent systems, including other fluorocarbon liquids, are available which may be advantageous for their viscosity, immiscibility with unusual analytes or sample solvents, or to match magnetic susceptibility to a particular sample.

Interfacing to Capillary Separation or Concentration. A variety of means have been proposed for microanalysis of trace samples by performing separation and concentration of sub-microliter volumes. Most of these systems are practical or viable in an openly accessible system on the benchtop, or within a specialized device. Most cannot be adapted to practice in the confined and inaccessible volume of an NMR magnet bore, and so cannot be used *in situ* for microcoil NMR as we demonstrated for capillary isotachophoresis. Nor has it previously been feasible to transfer the sub-microliter sample fractions produced by these methods into microcoil NMR probes. The immiscible solvent plug injection method makes it practical.

Fraction collection using ISPI. Traditional and present methods of flow NMR draw samples from microtiter plates such as 96-well plates with 200 uL wells. Arrays of smaller wells such as 384 well plates or 1536-well plates are also in use. A problem in automated sample handling is positioning a needle into the fluid sample volume and withdrawing a small sample completely without drawing any air. With small samples, a significant fraction of small samples must be left in the well, where it is wasted. By the

present method, rather than capturing and storing samples in the wells of microtiter plates, samples could be collected at the source of concentration or separation as immiscible plugs in a length of inexpensive teflon tubing filled with the immiscible carrier. The teflon tubing my be easily stored and/or transported to a different laboratory for microcoil NMR analysis or other microfluidic analytical methods.

Underlays and overlays The present invention also enables a more efficient method of handling small samples in conventional microtiter plates. To draw the entire prepared sample into an autosampler needle without drawing air, an immiscible fluid which is lighter (lower density) than the sample solvent may be added to the sample well together with the prepared sample. This lighter immiscible will float on top of the prepared sample. When the sample is drawn into the needle of the sample handling robot, any excess volume drawn will be the immiscible overlay rather than air, and the sample may be efficiently transferred into the microcoil NMR or other microfluidic device.

Relevant Literature:

** Behnia, B. and A. G. Webb (1998). "Limited-Sample NMR Using Solenoidal Microcoils, Perfluorocarbon Plugs, and Capillary Spinning." Anal. Chem. 70: 5326-5331.
Describes how samples bracketed by the fluorocarbon liquid FC43 may be much smaller than samples in air.

** Curcio, M. and J. Roeraade (2003). "Continuous segmented-flow polymerase chain reaction for high-throughput miniaturized DNA amplification." Anal Chem 75(1): 1-7.
Describes pumping sample plugs in fluorocarbon liquid through long lengths of Teflon tubing with low carryover between samples.

Nord, L. and B. Karlberg (1984). "Extraction Based on the Flow-Injection Principle. Part 6. Film Formation and Dispersion in Liquid-Liquid Segmented Flow Extraction Systems." Analytica Chimca Acta 164: 233-249.
Shows the phase with the highest affinity for the tubing material forms a thin film on the wall.

Kautz, R. A., M. E. Lacey, et al. (2001). "Sample Concentration and Separation for Nanoliter-Volume NMR Spectroscopy Using Capillary Isotachophoresis." J. Am. Chem. Soc. 123(13): 3159-3160.

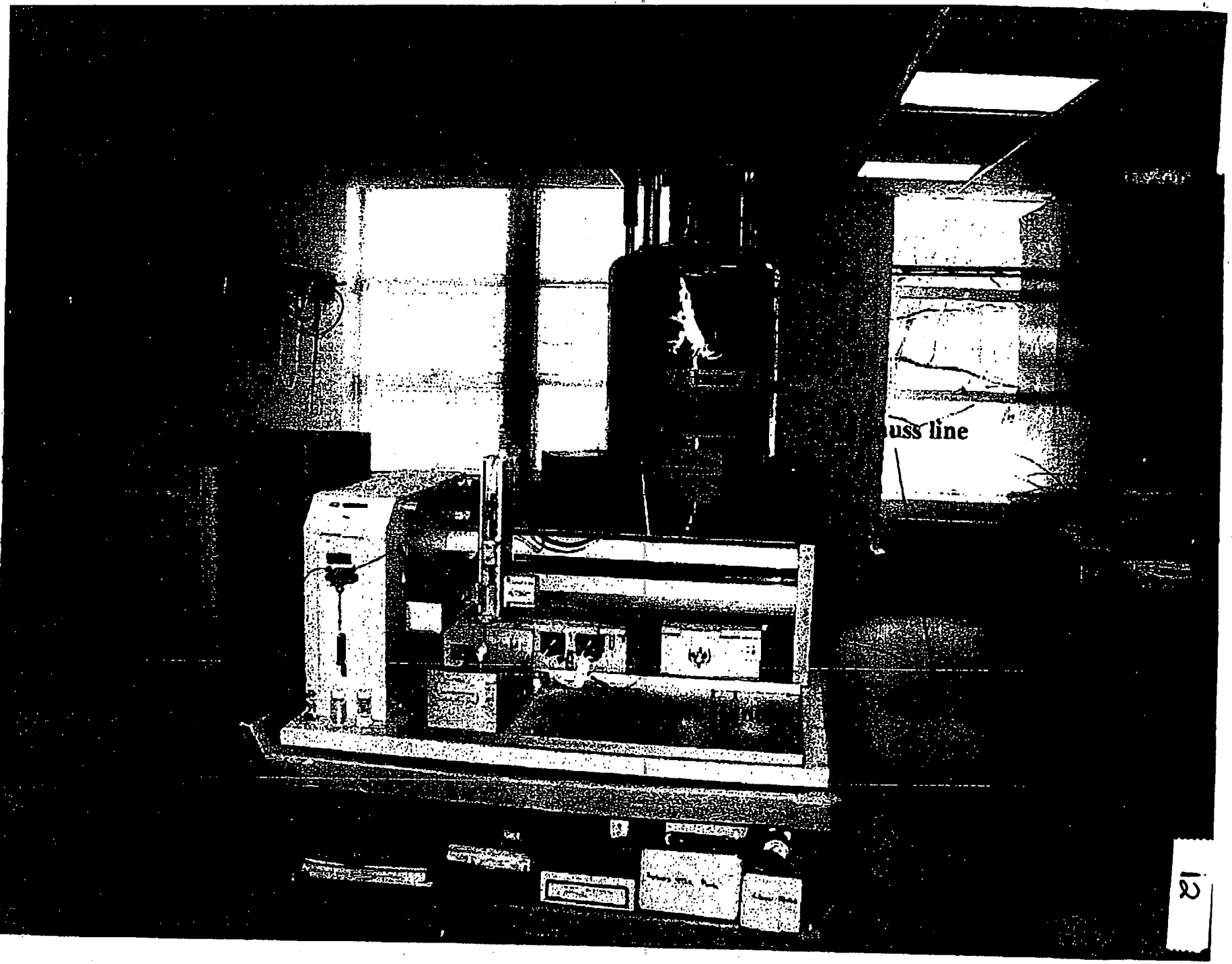
Keifer, Paul A. et al. (2000) "Direct Injection NMR (DI-NMR): A Flow NMR Technique for the Analysis of Combinatorial Chemistry Libraries" Journal of Combinatorial Chemistry 2(2):151-171.

Keifer, Paul A. (2003) "Flow Injection Analysis NMR (FIA-NMR): A Novel Flow NMR Technique that Complements LC-NMR and DI-NMR" Magnetic Resonance in Chemistry 41:509-516.

** Lacey, M. E., J. V. Sweedler, et al. (2001). "1H NMR Characterization of the Product from Single Solid-Phase Resin Beads Using Capillary NMR Flow Probes." J. Magn. Reson. 153(2): 215-222.
Describes use of fluorocarbon liquid to inject a sample into the flow cell of a microcoil NMR probe. Used manual injection with a syringe. Mentions it could be useful to automate.

Lacey, Michael E. et al. (1999) High-Resolution NMR Spectroscopy of Sample Volumes from 1 nL to 10 uL" Chemical Reviews 99:3133-3152. Review of microcoil NMR.

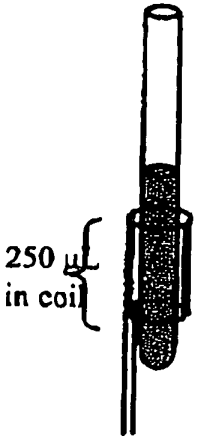
(Doc. no. 296792v.2)


uss line

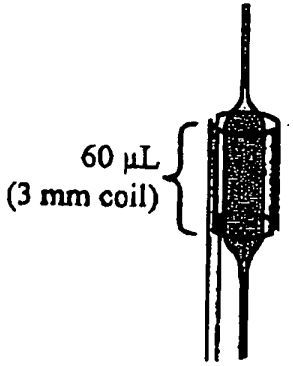
NMR Probes

Fig. 2

A: Conventional
5 mm Saddle Coil
(10 µg)

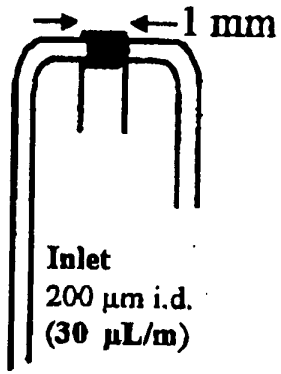


B: LC-NMR
3 mm Flow Probe
(3 µg)

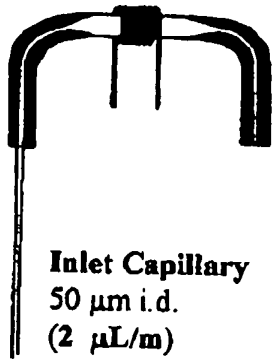


"Microcoil"
Solenoidal Capillary Coils

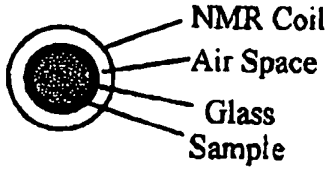
C: 200/360 micron capillary
30 nL observed Volume
(0.1 µg)

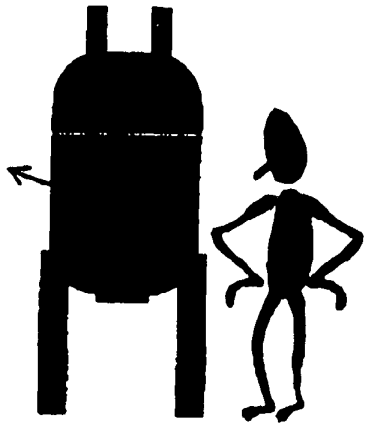


D: 1 mm flow cell
1 µL Observed Volume
(0.3 µg)



Cross-sectional view





Filling Factor: Fraction of coil volume occupied by sample.

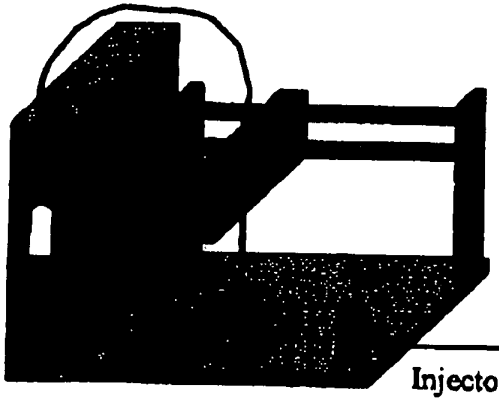
55 - 65%

75-85%

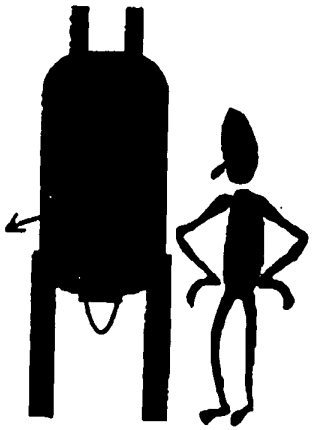
2% (50/370 µm capillary)
to 70% (450/540 µm capillary)

VAST High Throughput NMR

Fig. 3



Injector Port



Gilson 215
Sample Handler

Transfer Capillary
(2-5 meters)
125 µm
50 µm

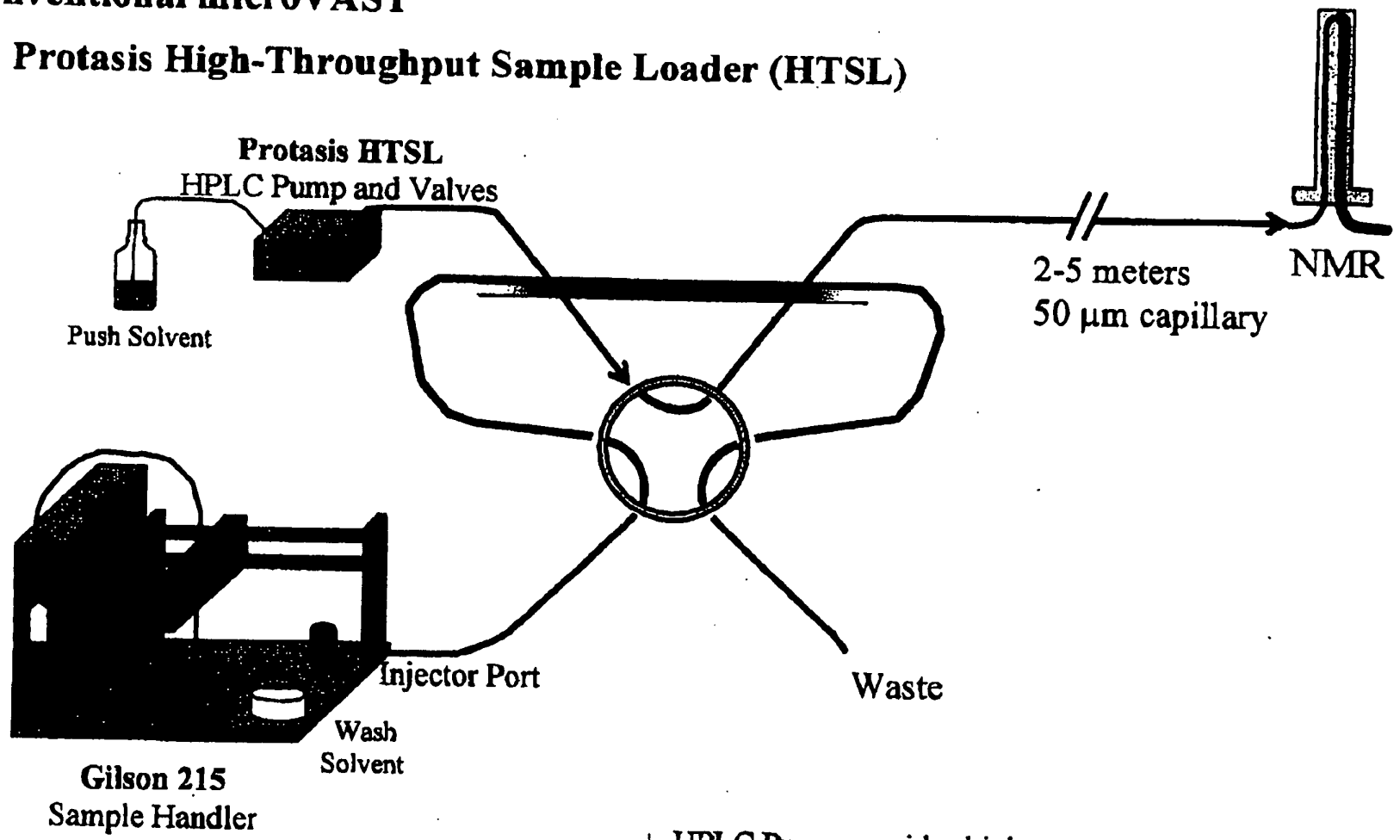
NMR Probe
150 uL Varian LC-NMR Probe
3 µL MRM capLC Flow Probe

VAST designed for 60 µL flow probe. 10-30 samples/hr.

With 3 µL MRM micro flow probe:

- Small 50 µm capillary required to minimize dispersion of sample plug.
- DMSO (solvent of choice) is viscous.
- Seal Failures at Injector Port due to High Back-Pressure.
- Long times (minutes) required to fill and to wash.

Conventional microVAST with Protasis High-Throughput Sample Loader (HTSL)



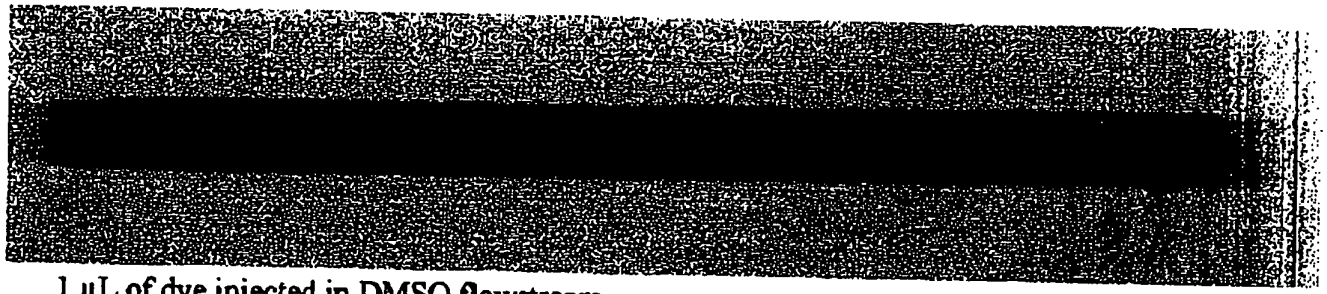
+ HPLC Pump provides higher pressure, more accurate positioning.
+ Injection valve/ loop eliminates high back-pressure on injector por

- Dispersion still a problem
- Relaxation time (1-3 min) after injecting each sample
- Need to fill/wash through entire system.

Injecting Sample as Immiscible Plug Avoids Dispersion

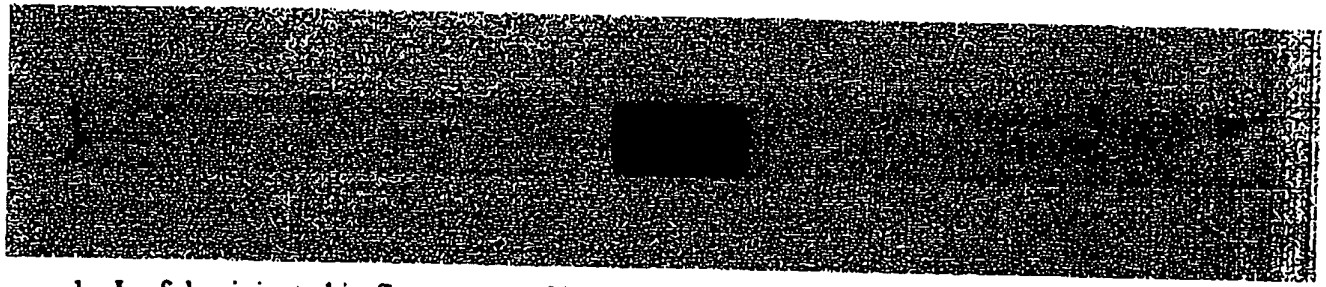
Fig. 5

A



1 μL of dye injected in DMSO flowstream.

B

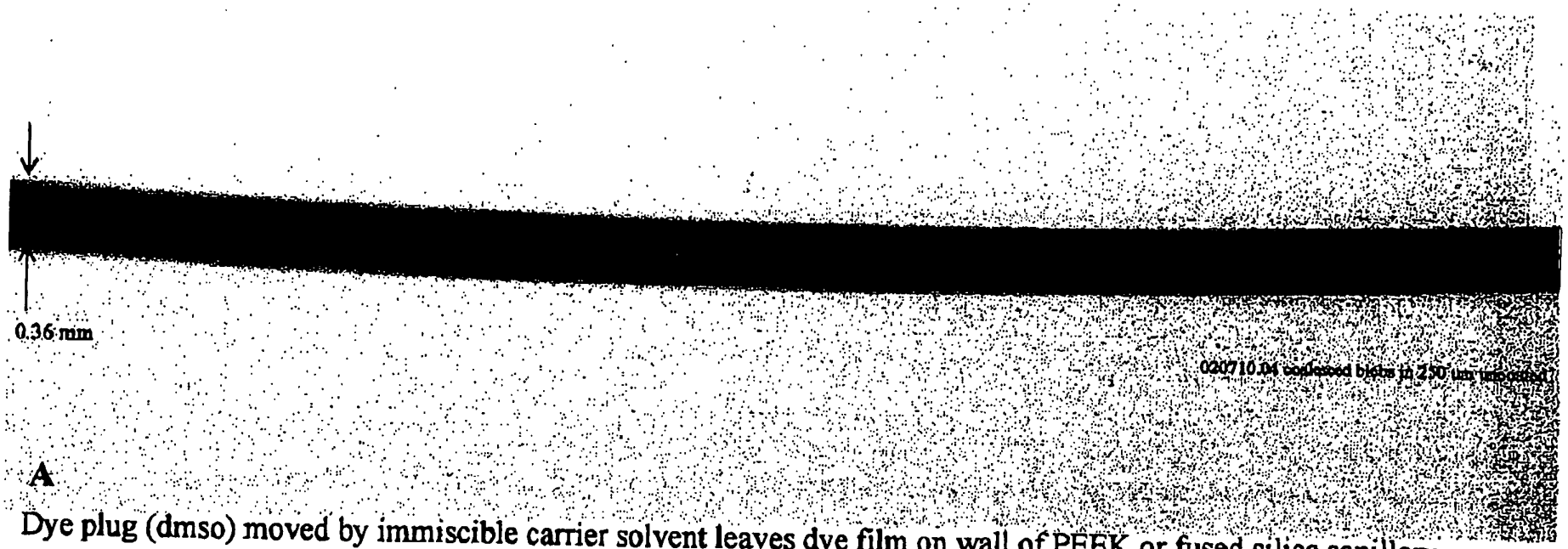


1 μL of dye injected in flowstream of immiscible fluorocarbon.

Notes: A 3x concentration increase means 10x faster data acquisition.
Can inject through large-bore capillary: lower back-pressure,
Faster relaxation.

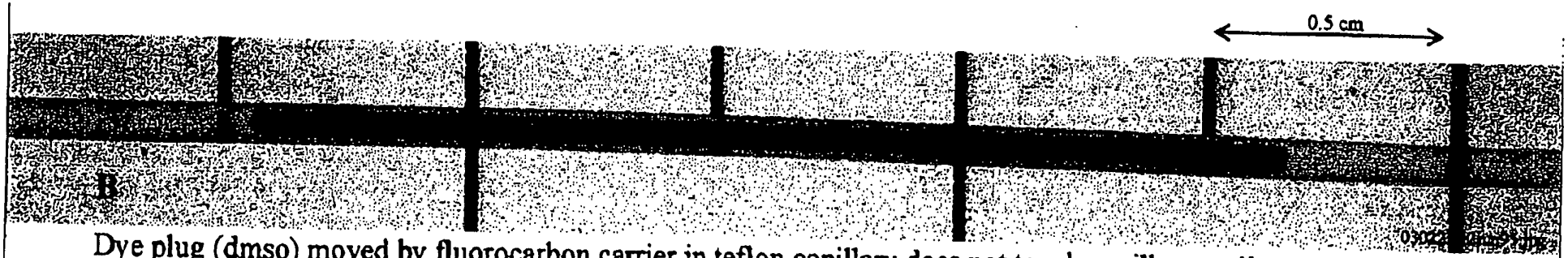
Lacey et al, J. Mag. Reson 153:215 (2001)
Behnia & Webb, Anal. Chem. 70:5326.

Fig. 6



A

Dye plug (dmso) moved by immiscible carrier solvent leaves dye film on wall of PEEK or fused silica capillary.

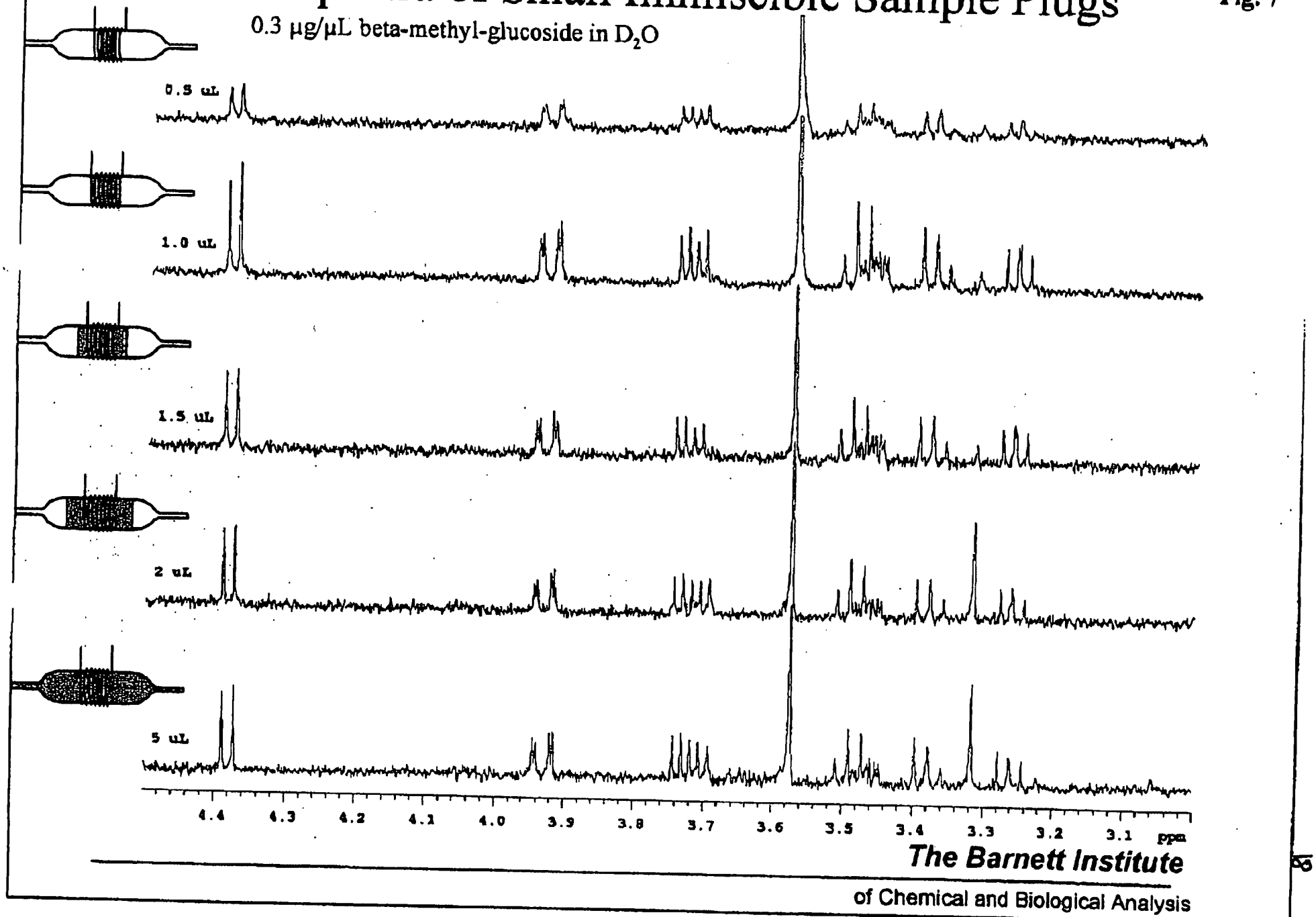


B

Dye plug (dmso) moved by fluorocarbon carrier in teflon capillary does not touch capillary wall.

NMR Spectra of Small Immiscible Sample Plugs

Fig. 7



The Barnett Institute

of Chemical and Biological Analysis

Immiscible Plug Injection for Microcoil NMR

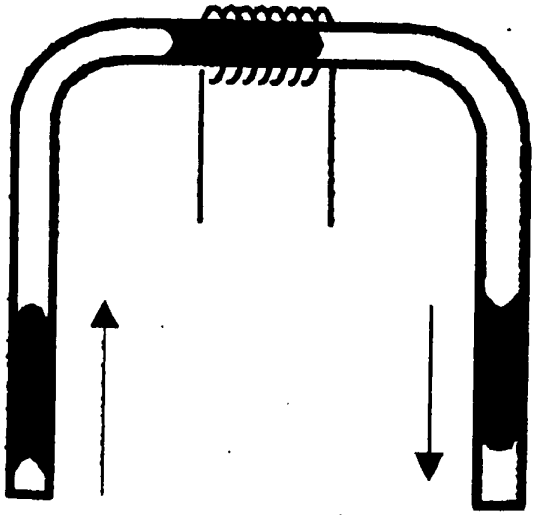
Fig. 8

Gives Higher Sample Concentration
for faster data acquisition

Conventional
1 μL → 5 μL

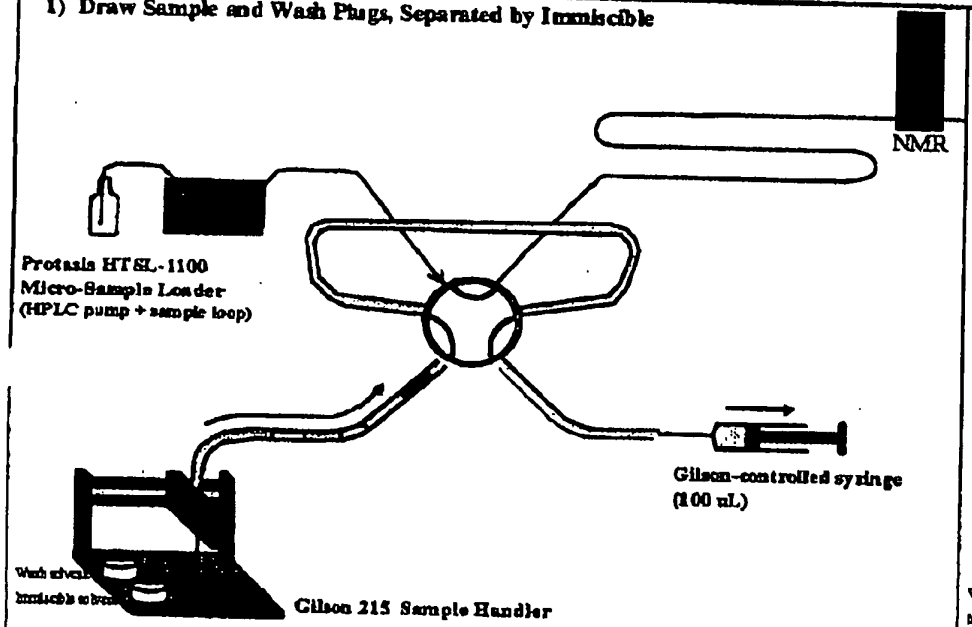
Immiscible
1 μL → 1 μL

Segmented, Interrupted Flow Injection
Can Queue Plugs, For Faster
Sample Changes and Washes

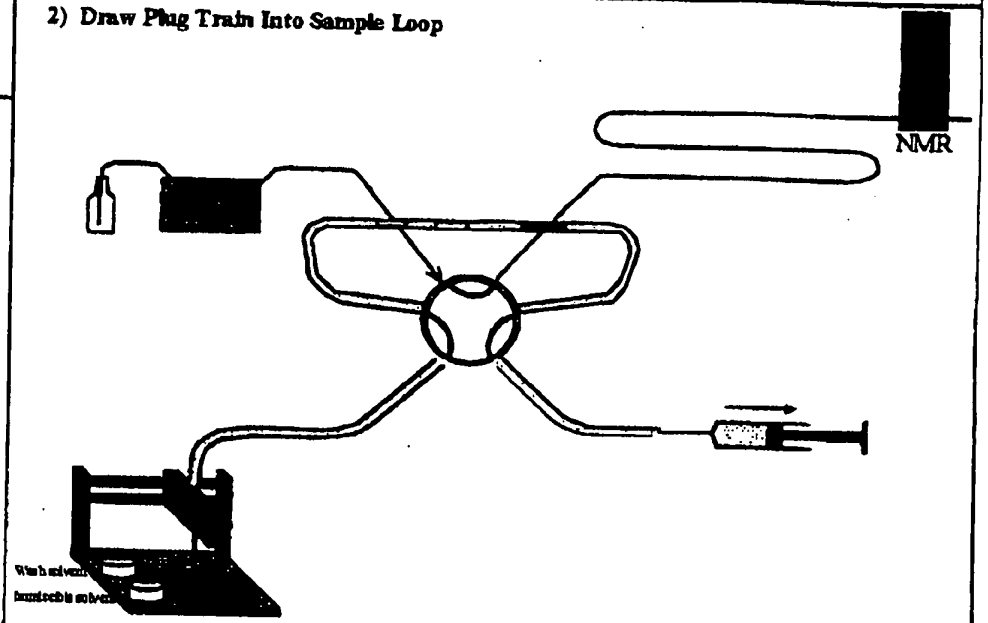


An Implementation of Immiscible Solvent Plug Injection NMR on Commercial Hardware. Fig. 9

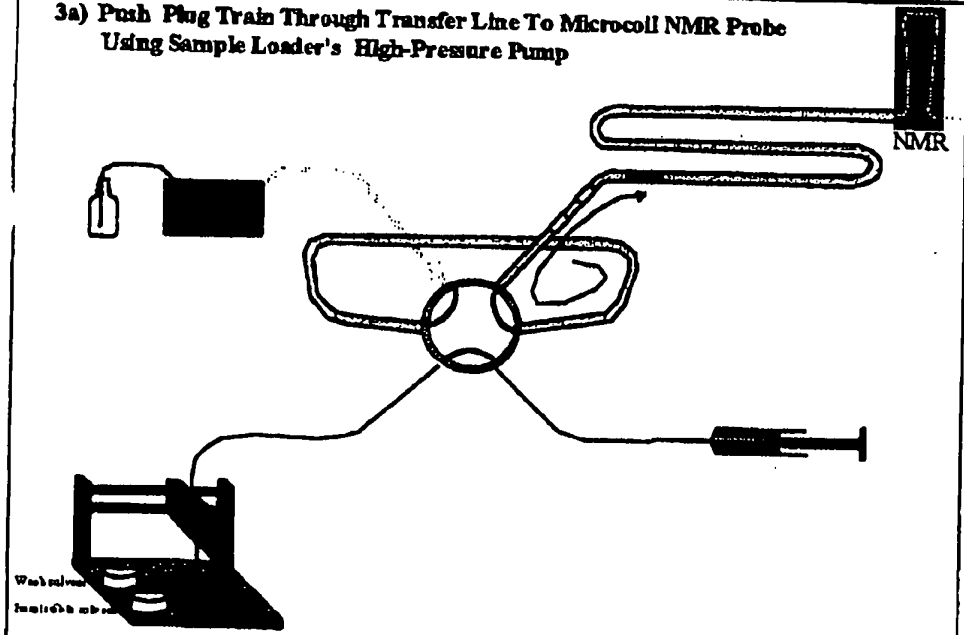
1) Draw Sample and Wash Plugs, Separated by Immiscible



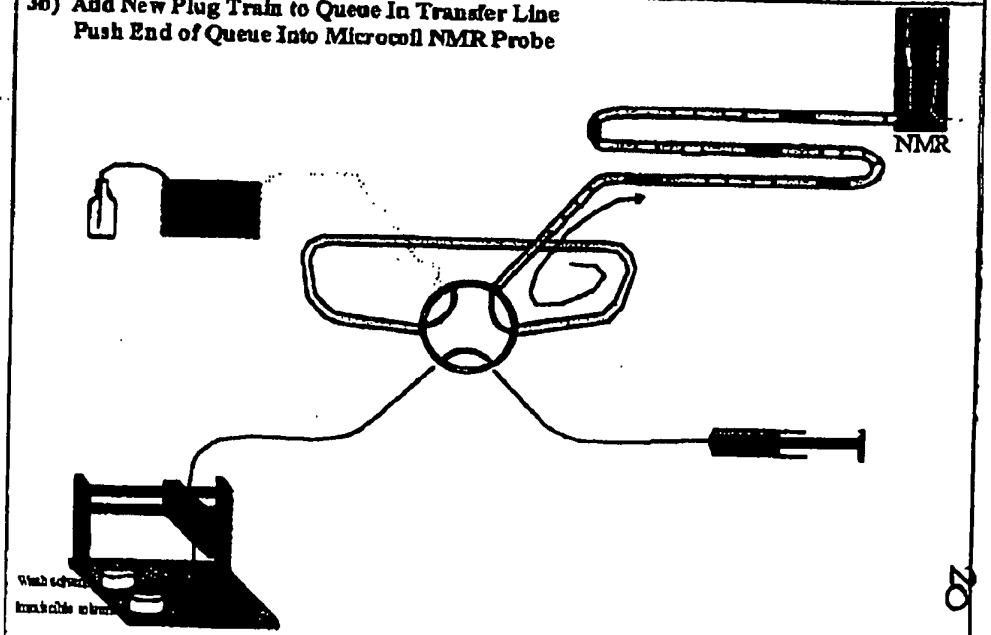
2) Draw Plug Train Into Sample Loop



3a) Push Plug Train Through Transfer Line To Microcoil NMR Probe Using Sample Loader's High-Pressure Pump



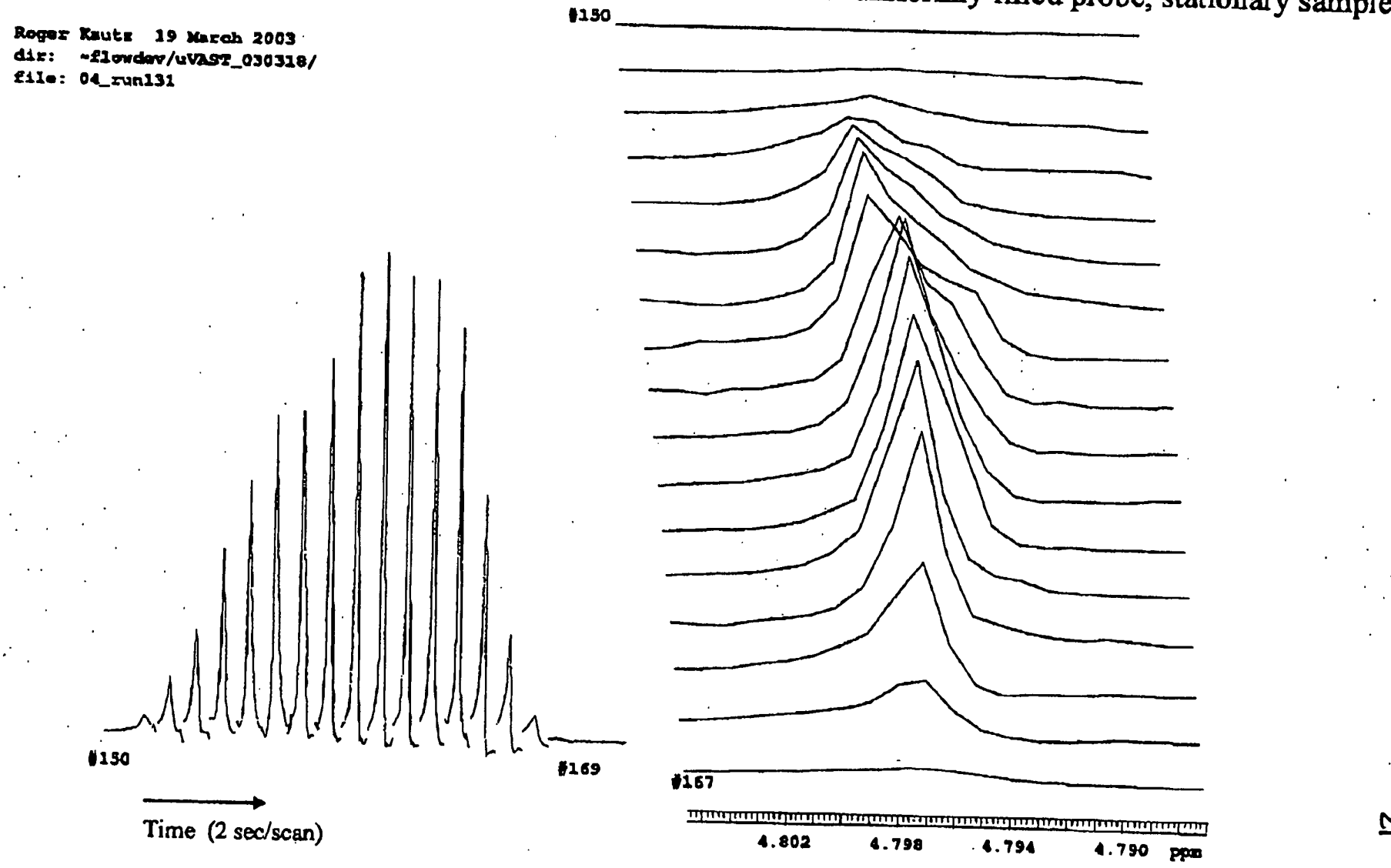
3b) Add New Plug Train to Queue In Transfer Line Push End of Queue Into Microcoil NMR Probe



2 uL DMSO plug in FC carrier solvent passing through 1 uL NMR detection coil at 3 uL/min

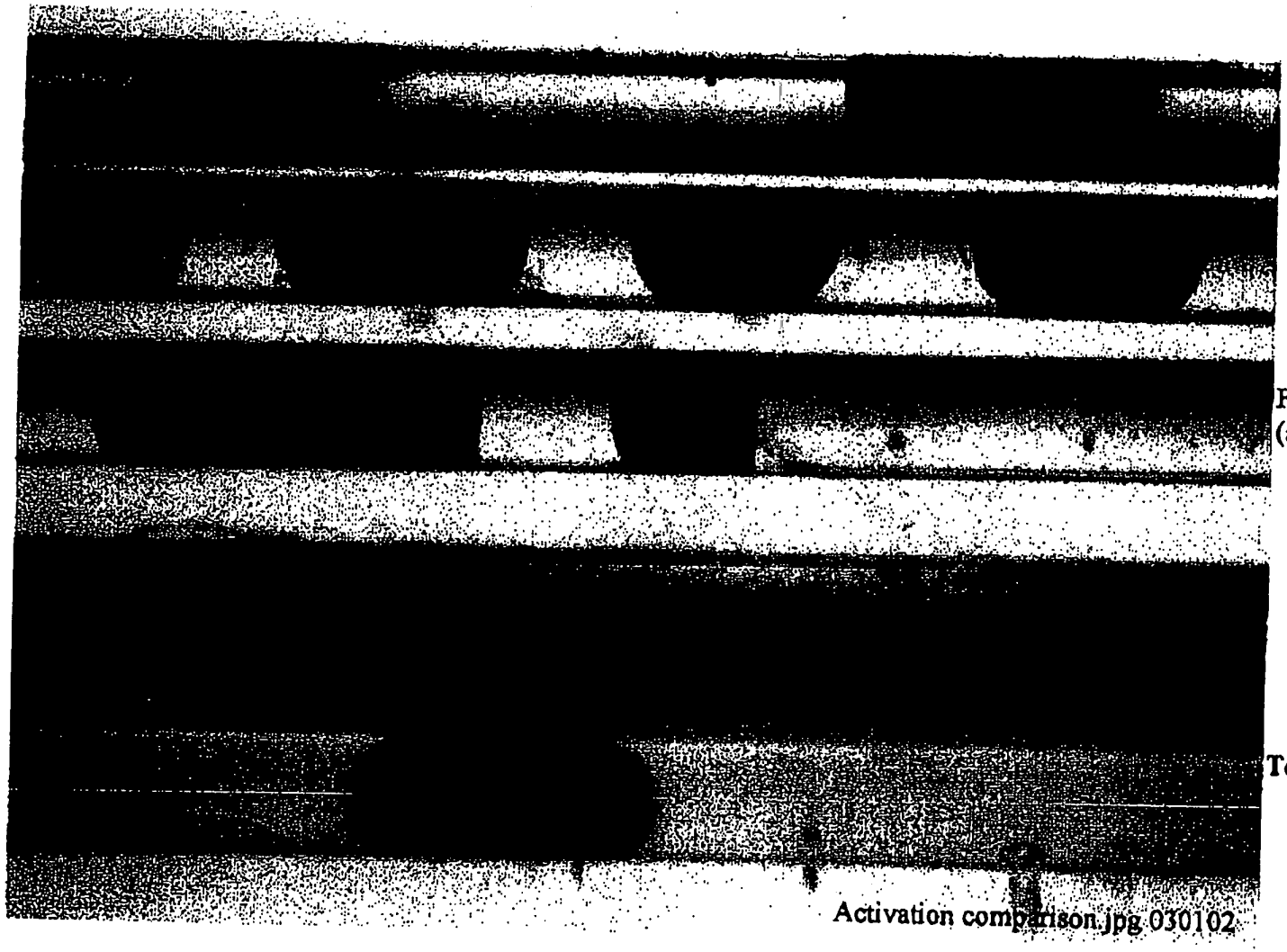
No "Relaxation Time" Required When Injecting Immiscible Plugs

Linewidths of plug passing on-flow are as good as uniformly filled probe, stationary sample



Comparison of plain fused silica, FAS-silica, and teflon tubing

Fig.11



Comparison of sample plugs (DMSO, 10 mg/mL of blue dye methyl green) with fluorocarbon FC-43 (clear) carrier fluid in the indicated tubing types. In plain fused silica the DMSO plugs are hourglass-shaped, as the sample wets the bare silica wall preferentially over the fluorocarbon; smear at left is coalesced from film of sample retained on wall as plug was moved into its current position. DMSO plugs in teflon are sausage-shaped; tangential contact angle with wall indicates wall is wetted by fluorocarbon. Perfluoro-alkyl silane (FAS) coated silica capillaries also favor wetting by fluorocarbon; moving DMSO plugs do not leave film, but can touch wall if left stationary.

Document made available under the Patent Cooperation Treaty (PCT)

International application number: PCT/US04/041923

International filing date: 10 December 2004 (10.12.2004)

Document type: Certified copy of priority document

Document details: Country/Office: US
Number: 60/528,410
Filing date: 10 December 2003 (10.12.2003)

Date of receipt at the International Bureau: 24 January 2005 (24.01.2005)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b)

World Intellectual Property Organization (WIPO) - Geneva, Switzerland
Organisation Mondiale de la Propriété Intellectuelle (OMPI) - Genève, Suisse

This Page is Inserted by IFW Indexing and Scanning Operations and is not part of the Official Record.

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ BLACK BORDERS
- ☐ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES
- ☐ FADED TEXT OR DRAWING
- ☐ BLURRED OR ILLEGIBLE TEXT OR DRAWING
- ☐ SKEWED/SLANTED IMAGES
- ☐ COLOR OR BLACK AND WHITE PHOTOGRAPHS
- ☐ GRAY SCALE DOCUMENTS
- ☑ LINES OR MARKS ON ORIGINAL DOCUMENT
- ☐ REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY
- ☐ OTHER: _____

IMAGES ARE BEST AVAILABLE COPY.
As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.